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Electrofishing Injury and Short-Term Mortality in Hatchery-Reared Rainbow Trout Stocked into an Ozark Stream

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Abstract.—We conducted an electrofishing injury study to evaluate potential effects of sampling procedures on survival and health of hatchery rainbow trout *Oncorhynchus mykiss* (187–307 mm total length) stocked into an Ozark stream. We assessed two groups of trout: one group had acclimated to stream conditions for 1 month; the other group was handled and transported just before the study. Each group was sampled by electrofishing (boat-mounted, 60-Hz AC) and seining (controls), resulting in four treatment groups (N = 21). We held fish for 48 h to evaluate mortalities in that period, then euthanized all fish and examined them for hemorrhages and spinal damage. No fish died during the 48-h holding period, indicating that our procedures did not cause significant sampling-related mortality among stocked trout in Brush Creek. Spinal damage was observed in 5% of fish collected with electrofishing but in none of the control fish. We found hemorrhages in 90% of electrofished trout but none in control fish, and recently handled and hauled trout had a greater occurrence and severity of hemorrhaging.

Electrofishing techniques may cause trauma to rainbow trout *Oncorhynchus mykiss* produced in the wild (Sharber and Carothers 1988; Thompson et al. 1997a; McMichael et al. 1998) and in hatcheries (Hudy 1985; McMichael 1993; Schill and Elle 2000). Electrofished fish may show no external sign of damage but may sustain internal injuries, including hemorrhages in musculature and spinal compression or fracture (Reynolds 1996). A nominal rating system for common electrofishing injuries (hemorrhaging and spinal damage; Reynolds 1996) and standardized procedures to evaluate electrofishing injury (Reynolds and Holliman 2000) have facilitated study of deleterious effects of electrofishing on fish in recent years. Standardized procedures include sampling 20 fish of similar size by using electrofishing and collecting 20 control fish by another method, such as seining or angling (Reynolds and Holliman 2000). All fish collected are then euthanized and filleted on both sides to evaluate the presence and severity of hemorrhaging according to the rating system of Reynolds (1996). If high numbers of hemorrhages are observed, it is advisable to examine fish by radiography to evaluate the presence and severity of spinal damage, again by the rating system of Reynolds (1996). Reynolds and Holliman (2000) recommended that both dorsal and lateral perspective X-rays be performed to evaluate both presence and directionality of spinal injuries.

As part of a 3-year study to evaluate potential effects of rainbow trout introduction on native fish populations in a northeastern Oklahoma Ozark stream, we stocked hatchery-reared rainbow trout into a stream November 2000–March 2001 and November 2001–March 2002. We established standardized electrofishing procedures (60-Hz AC, 3–4 A) for sampling native fish assemblages in the year before we started trout stocking. To allow for comparisons of electrofishing data among years, we did not alter these electrofishing specifications after trout were introduced. No fishing took place in this system, so we depended on electrofishing methods to sample stocked trout to evaluate movement, habitat use, and survival.

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We sampled rainbow trout frequently, including sampling the stocking site on the same day that trout were stocked. However, we were concerned that sampling-related mortality from electrofishing could bias our survival estimates and influence the results of our research. To evaluate the effects of our electrofishing methods on the survival and health of stocked rainbow trout in our study stream, we conducted an electrofishing injury experiment in December 2001. Our objectives were to (1) estimate short-term (48 h) mortality in stocked rainbow trout sampled by electrofishing; (2) evaluate sublethal injuries (hemorrhages and spinal damage) in these fish; and (3) evaluate whether recently handled and transported fish were more susceptible to sampling-related mortality or injury than those that had been stocked 1 month earlier.

Methods

Experimental design.—Our electrofishing injury study evaluated potential effects of electrofishing sampling procedures on survival and health of hatchery rainbow trout stocked into an Ozark stream. We assessed two groups of trout: one group (stocked in November 2001) had acclimated to stream conditions for 1 month; the other group (stocked in December 2001) was handled and transported just before the study. Because we sampled each group by electrofishing (boat-mounted, 60-Hz AC) and seining (controls), we obtained four treatment groups: control/stocked in November 2001, control/stocked in December 2001, electrofished/stocked in November 2001, electrofished/stocked in December 2001. We allocated 21 fish to each treatment group, based on the recommendation of Reynolds and Holliman (2000) that including at least 20 similarly sized fish in each treatment group represents a sufficient sample size to evaluate electrofishing injuries.

Stocking and sampling.—We stocked 500 individually marked rainbow trout into Brush Creek, Delaware County, Oklahoma, in November 2001 and again in December 2001. This experiment was conducted immediately after stocking in December 2001. Neither group had been electrofished before this experiment. Before stocking, all rainbow trout were anesthetized with tricaine methanesulfonate (MS-222), measured for total length (TL, mm), weighed (g), and tagged with Floy FD-68B anchor tags and visual implant elastomer (VIE) tags at Crystal Springs Trout Farm, Cassville, Missouri. After the trout remained in the raceway at the hatchery overnight, they were loaded and hauled for approximately 3 h to the stocking site at Brush Creek.

Brush Creek is a small (mean width, 9 m), spring-fed stream in Delaware County, Oklahoma; it originates at a large natural spring and flows approximately 10 km before draining into Lake Eucha. The stocking site is a large (710 m^2) bedrock-formed lateral scour pool with fractured bedrock cover; average depth is 1.16 m and maximum depth 2 m. Before stocking it in December 2001, we blocknetted the site to prevent movement of stocked fish before electrofishing. After stocking, we seined control fish, then electrofished the stocking site (13°C, 336 μS/cm), using a boat-mounted Smith-Root 2.5 GPP electrofisher as part of our standard sampling procedure (three passes at 60-Hz AC, 3–4 A) to estimate the proportion of stocked trout remaining in the pool. During sampling, all electrofished rainbow trout were collected, and 21 fish from each stocking group collected in the final pass were randomly selected for use in our experiment. To allocate fish into treatment groups, we identified the stocking date of each fish (November or December 2001) by Floy tag number and color.

We anesthetized, weighed, and measured all electrofished and control fish (N = 21 per group) and distributed the fish among 12 net pens (three pens per group, seven trout per pen) in the stream and checked for mortality after 24 and 48 h. All fish still alive after 48 h were euthanized with a lethal concentration of MS-222 and transported on ice to Oklahoma State University for evaluation of hemorrhaging and spinal damage.

Injury evaluation.—We followed guidelines by Reynolds and Holliman (2000) to evaluate presence and severity of hemorrhaging and spinal damage. To evaluate hemorrhaging, we filleted fish, examined both the body and fillets for hemorrhages, and categorized perceived severity according to the worst hemorrhage found: Level 0 = none apparent; Level 1 = one or more small wounds in the muscle; Level 2 = one or more small (no more than the width of two vertebrae) wounds on the spine; Level 3 = one or more large (more than the width of two vertebrae) wounds on the spine (Reynolds 1996). For evaluation of spinal damage after filleting, all fish were imaged by X-ray at the OSU Veterinary Teaching Hospital (lateral perspective only) with a Picker GX550 single-phase X-ray unit (52 kilovoltage peak [kVp], 500 mA, 0.5-s exposure time, X-O mat TL Kodak nonscreen film) at a distance of 102 cm. We categorized spinal damage according to Reynolds (1996): Level

TABLE 1.—Mean length and percent of rainbow trout with hemorrhages and spinal damage among treatment groups sampled from Brush Creek to evaluate electrofishing injury in December 2001.

Treatment	Month stocked	N	Mean length (SD; mm)	Percent injured	
				Hemorrhage	Spinal damage
Control	Nov	21	256 (17)	0	0
	Dec	21	240 (12)	0	0
Electrofished	Nov	21	259 (30)	81	5
	Dec	21	244 (22)	100	5

0 = none apparent; Level 1 = compression of vertebrae only; Level 2 = misalignment of vertebrae, including compression; Level 3 = fracture of one or more vertebrae or complete separation of two or more vertebrae.

Analysis of electrofishing injuries.—We calculated the proportions of fish with each injury type (hemorrhage or spinal damage) within each treatment group and the proportions of fish with injury at each level of injury severity, as suggested by Reynolds and Holliman (2000). We calculated Cochran–Mantel–Haenszel (CMH) statistics to evaluate the relationship between each injury type (hemorrhages and spinal damage) and electrofishing, controlling for the month in which the fish were stocked (Proc FREQ, SAS 2000).

To evaluate severity of hemorrhaging, we used a baseline category logit model for multinomial, nominal responses (Agresti 2002). Reynolds and Holliman (2000) specify that the categories of injury severity should not be treated as ordinal. We set the baseline category to Level 0 (no injury) to facilitate comparison and used parameter estimates to calculate the odds of rainbow trout suffering each injury level relative to suffering no injury. For both CMH and logit analyses, we added 0.5 to all cell values to allow calculation of odds ratios and maximum-likelihood estimators of model parameters (Agresti 2002).

Analysis of fish length.—We used a two-way analysis of variance (ANOVA) to evaluate relationships between the dependent variable, length, and independent variables representing (1) the month when trout were stocked and (2) whether or not fish were electrofished ($\alpha = 0.05$; Proc GLM, SAS 2000).

TABLE 2.—Percent of rainbow trout with hemorrhages (according to perceived severity), among treatment groups sampled from Brush Creek to evaluate electrofishing injury in December 2001.

Treatment	Month stocked	N	Perceived severity (percent)			
			0	1	2	3
Control	Nov	21	100	0	0	0
	Dec	21	100	0	0	0
Electrofished	Nov	21	19	5	67	10
	Dec	21	0	0	86	14

Results

We observed no mortalities among electrofished or control treatment groups after 24 and 48 h. Electrofished rainbow trout had high levels of hemorrhaging and low levels of spinal damage, regardless of the amount of time spent in the stream before they were sampled by electrofishing (Table 1). We did not observe similar muscular or spinal damage in control fish. Cochran–Mantel–Haenszel statistics indicated that occurrence of hemorrhaging increased when fish were electrofished (CMH = 66.22, df = 1, $P < 0.0001$), whereas occurrence of spinal damage was not related to whether fish were electrofished (CMH = 1.02, df = 1, P = 0.3116).

Just one rainbow trout in each electrofishing treatment group exhibited spinal injury (both Level 1), indicating overall low occurrence (5%) and severity of spinal damage; no control fish had spinal damage (Table 1). Hemorrhaging occurred in 81% of rainbow trout stocked in November 2001, and 100% of trout stocked in December 2001 (90% in both electrofishing treatment groups combined; Table 1). Severity level of hemorrhaging was usually Level 2 (Table 2). Rainbow trout that were electrofished were 17.7 times more likely than control fish to suffer a Level 1 hemorrhage (controlling for time spent in stream; Table 3). Estimated odds of electrofished rainbow trout sustaining Level 2 or 3 hemorrhages were 426.7 and 76.2 times greater, respectively, than control fish (Table 3).

Recently handled and transported fish appeared to be more susceptible to hemorrhaging than those stocked 1 month earlier. No trout stocked in December had Level 0 or 1 injuries; all had Level 2 or 3 hemorrhages (Table 2). Additionally, the mean number of hemorrhages per fish was greater for fish stocked in December (9.3) than for fish stocked in November (6.2; F = 10.53, model df

TABLE 3.—Parameter estimates and standard errors (in parentheses) for generalized logit model fitted to hemorrhage occurrence and severity data for rainbow trout stocked into Brush Creek. Odds of each injury level relative to no injury (Level 0) are calculated as e^x, where x = the parameter estimate for each level (for example, $e^{2.876} = 17.7$ for Level 1 electrofished rainbow trout).

Parameter	Hemorrhage severity categories for logit		
	Level 1/Level 0	Level 2/Level 0	Level 3/Level 0
Intercept	−3.819 (1.238)	−4.720 (1.254)	−4.666 (1.337)
Month stocked (Dec = 1)	0.1160 (1.387)	1.461 (0.929)	1.393 (1.111)
Electrofishing treatment (Yes = 1)	2.876 (1.368)	6.056 (1.231)	4.334 (1.287)

= 1, error df = 40, corrected total df = 41, P = 0.0024). Fish recently handled and transported were 1.1 times more likely than fish that had acclimated to the stream for 28 d to sustain a Level 1 hemorrhage (controlling for electrofishing treatment; Table 3). Estimated odds of fish stocked in December 2001 suffering Level 2 or 3 hemorrhages were 4.3 and 4.0 times greater, respectively, than fish stocked in November 2001 (Table 3).

Our electrofishing sample contained subsamples of fish that had been stocked in both November (207–307 mm) and December 2001 (187–277 mm). Control fish stocked in November ranged from 229 to 298 mm and those stocked in December ranged from 214 to 255 mm. Mean lengths were significantly different among treatment groups (F = 3.85, model df = 3, error df = 80, corrected total df = 83, P = 0.0125). Length of fish in each treatment group was dependent on the month when the trout were stocked (F = 11.04, df = 1, P = 0.0013). Trout stocked in November 2001 (mean ± SD; 257 ± 24 mm) were larger than those stocked in December 2001 (242 ± 18 mm). No difference in length was detected between electrofished (251 ± 27 mm) and control fish (248 ± 16 mm; F = 0.52, df = 1, P = 0.4750). The interaction between month stocked and electrofishing was not significant (F = 0.00, df = 1, P = 0.9837), indicating that only the month of stocking contributed to differences in lengths among treatment groups. The sizes of rainbow trout stocked in each month reflected the size distribution of fish available at the hatchery in each month. Considering all trout stocked into the stream, trout stocked in November 2001 (253 ± 25 mm) were larger than those stocked in December 2001 (244 ± 22 mm; F = 35.54, model df = 1, error df = 1045, corrected total df = 1046, $P > 0.0001$).

Discussion

Despite 81–100% hemorrhaging in electrofished rainbow trout, we did not observe any mortalities after 48 h. Other studies have also shown high incidence of hemorrhage with low mortality (McMichael 1993; Schill and Elle 2000), reflecting the ability of fish to survive injuries such as hemorrhaging. The hemorrhage levels we observed were similar to those observed by Schill and Elle (2000; 81.6–86.1%), who used DC electrofishing on similarly sized trout at similar conductivity in a hatchery setting. As many as 10 hemorrhages per fish occurred among fish in our electrofishing treatment groups, exceeding the maximum number of hemorrhages (5) per fish recorded by Schill and Elle (2000).

Rainbow trout stocked in December 2001 that were handled and transported just before electrofishing exhibited higher occurrence and severity of hemorrhages than trout stocked in November 2001, which had acclimated to stream conditions for 28 d. Larger fish size is generally associated with more severe electrofishing injury (Hollender and Carline 1994; McMichael et al. 1998), but in this case smaller fish (those stocked in December 2001) exhibited higher hemorrhage occurrence and severity. We hypothesize that stress from recent handling and hauling may have made these fish more susceptible to injury and contributed to the greater hemorrhaging observed in this group. The time that we electrofished after stocking generally corresponded to the peak time of stress response after handling and hauling (3 h; Wagner et al. 1997). However, we did not directly evaluate stress on the fish and cannot conclusively link the stress of handling and hauling to increased occurrence and severity of hemorrhaging.

We found little evidence of spinal damage in electrofished rainbow trout, regardless of time spent in the stream before sampling. Other studies using AC electrofishing have also found low incidence of spinal damage (Hudy 1985; Habera et al. 1996). Using DC, Dalbey et al. (1996) observed 37% spinal damage on rainbow trout similar in size to those in this study. Greater rates of spinal damage have been found in larger rainbow trout obtained by AC (Sharber and Carothers 1988) and

DC (Sharber et al. 1994; Thompson et al. 1997a) electrofishing methods. Spinal injury occurrence or severity may have been underestimated in this study because we were able to conduct only lateral perspective X-rays. McMichael et al. (1998) found that lateral X-rays detected 87% of spinal injuries to stream salmonids; 13% of injuries were detected only with dorsal views. Lateral X-rays alone are often sufficient to detect the presence of spinal injury, but including dorsal perspective X-rays may allow for more accurate classification of injury severity (Thompson et al. 1997a; Habera et al. 1999). Because of the additional costs of radiograph procedures, we wanted to determine whether hemorrhages were present before deciding to perform X-rays. Once filleted and defrosted, however, the carcasses were too fragile for us to perform dorsal perspective X-rays. Given the nature of the ongoing research project, we did not wish to remove an additional 80 stocked rainbow trout from the stream to perform dorsal perspective X-rays. Therefore, we may have underestimated spinal injury severity because we were unable to detect directional injuries.

Hemorrhaging may have less implications on long-term effects to salmonids than does spinal damage (Habera et al. 1999). Schill and Elle (2000) documented 78% reduction in hemorrhages 5 weeks after electrofishing. Hemorrhage may be a short-term injury from which fish can recover with no ill effects. However, electrofishing may negatively affect the growth of rainbow trout (Dwyer and White 1995; Dalbey et al. 1996; Thompson et al. 1997b), and energy allocated to healing electrofishing-induced hemorrhages may further reduce that available for growth. Rainbow trout stocked into Brush Creek acclimated slowly to eating a natural diet and consumed low numbers of prey items (Fenner 2002); energetic effects of injury and healing could be particularly detrimental to recently stocked rainbow trout. The low mortality and spinal damage that we observed indicated that our sampling methods did not contributing to mortality of stocked trout that might bias survival estimates. The incidence of hemorrhaging that we observed may be cause for concern; however, it is unclear how these injuries may influence long-term survival and health of stocked rainbow trout.

Conclusions

Our results allow us to make some recommendations regarding electrofishing methods for sampling hatchery-reared rainbow trout stocked into natural warmwater streams. Reynolds and Holliman (2000) suggest that greater than 10% injury may be cause for concern, depending on specific management goals. Our observed spinal damage of 5% falls below this guideline, whereas our observed incidence of hemorrhaging (90%) exceeds it. Given the relatively rapid healing rate of hemorrhages (Schill and Elle 2000) and the low levels of spinal damage observed, occasional sampling to monitor population size or movement of stocked trout should not bias survival estimates in Ozark highland streams. Additionally, because of the increased occurrence and severity of hemorrhaging in recently stocked trout, we recommend allowing time for stocked rainbow trout to acclimate to stream conditions before electrofishing.

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